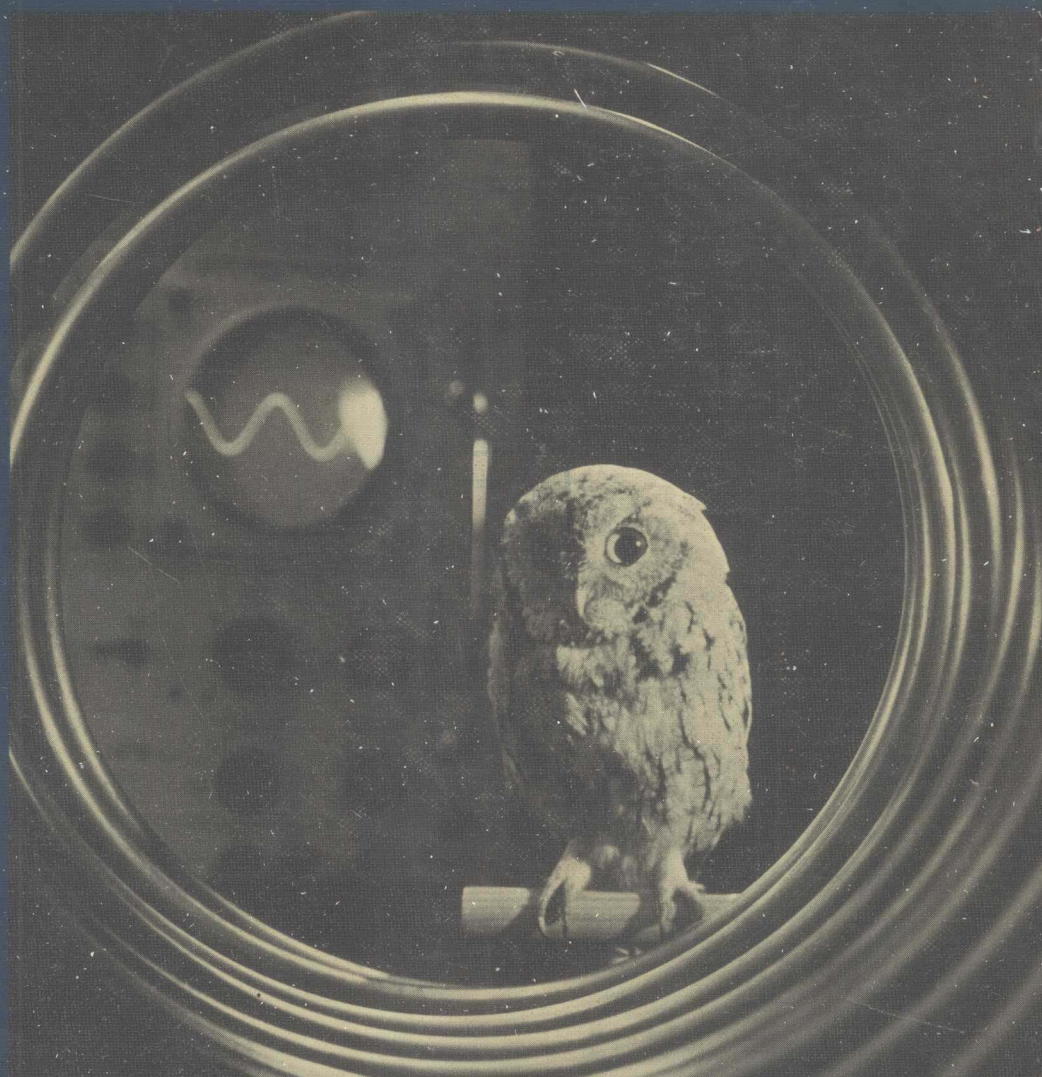


Freeze-Drying Biological Specimens: A Laboratory Manual

ROLLAND O. HOWER



Freeze-Drying Biological Specimens: A Laboratory Manual

By Rolland O. Hower
Office of Exhibits Central
Smithsonian Institution
Washington, D.C.

Introduction by
R. H. Harris
British Museum of Natural History
London, England

Copyright © 1979 Smithsonian Institution. All rights reserved.

Library of Congress Cataloging in Publication Data

Hower, Rolland O.

Freeze-Drying Biological Specimens: A Laboratory Manual.

Bibliography: p.

Includes indexes.

1. Freeze-drying. 2. Biological specimens—

Collection and preservation. I. Title.

QH324.9.C7H68 579.2 78-10750

ISBN 0-87474-532-2

Cover: Freeze-dried specimen of Maxwell screech owl (*Otus maxwellii*)

Photograph by James A. Mahoney, Chief, Office of Exhibits Central,
Smithsonian Institution.

All other photographs by the author.

Typeface: Times Roman

Paper: Paloma 70 lb. text, coated matte

Designed by Natalie Babson

Printed by Eastern Press, Inc.

CONTENTS

Foreword by Paul N. Perrot	13
Preface: A Brief Survey of Freeze-Dry Research	15
Introduction: by R. H. Harris	19
I. FUNDAMENTALS OF FREEZE-DRY	23
Freezing Biological Tissue	23
Thermodynamics of Water: Its Properties in Various States	25
Vapor Pressure and Sublimation	26
The Freeze-Dry Concept	28
Transfer of Water Molecules to Specimen Surface	29
II. ENGINEERING SPECIFICATIONS	31
Basic Requirements of a Freeze-Dry System	31
Vacuum Pump	31
Rotary Oil Pump	32
Rotary Piston Pump	33
Gas Ballasting	34
Oil Diffusion Pump	35
Calculating Pump Capacity	35
Sealing Oil	37
Vacuum-Line Dimensions	37
Vacuum Measurement	39
Basic Concepts of Refrigeration	42
Refrigerants	43
Expansion Valves	44
Thermal Insulation	45
Temperature Measurement	46
Simple Freeze-Dry Apparatus in a Chest-Type Freezer	47
Condenser	48

Freeze-Dry System with a Refrigerated Specimen Chamber	49
Large Freeze-Dry System	51
Apparatus for Freeze-Drying Cell Material	53
III. BIOLOGICAL APPLICATIONS	55
Preparation of Zoological Specimens	55
Wire for Mounting Specimens	55
Small Mammals	56
Birds	58
Collecting Zoological Specimens	58
Labels and Notes	62
Cleaning Feathers	62
Stopping Blood Flow in Fresh Zoological Specimens	63
Reptiles	63
Fishes	66
Marine Invertebrates	68
Eyes	72
Insects and Insect Larvae	74
Killing the Larvae	82
Killing Arachnids	82
Fresh Anatomical Specimens	83
Preserved Biological Specimens	88
Processing a Human Fetus	89
Processing a Human Brain	96
Pathology Specimens	100
Microanalytic Study of Inflated Whole Lungs	100
Protecting Specimens from Insects	107
Shipping Frozen Biological Specimens	108
Preparation of Specimens for Scanning Electron Microscopy	109
Ostracods	110
Metal Coating	112
Nematodes	117
Human Brain Material	121
Preservation of Marine Archeological Materials	124
Salted Beef	124
Electrolytic Conduction During Freezing	125
IV. APPENDIX A Drying Rate Charts	131
APPENDIX B Temperature Conversion Tables	173
V. GLOSSARY	177
VI. REFERENCES	183
VII. INDEX A Authors	190
INDEX B Subject	191

Figures

1. Pallas Cat, <i>Felis manul</i>	Frontispiece
2. Elementary Schematic of a Freeze-Dry System	31
3. Rotary Oil Pump	33
4. Rotary Piston Pump	33
5. Schematic of Gas Ballast	34
6. Vacuum Pump-Down Factors	36
7. Vapor-Line Conductance	39
8. Refrigerant Characteristics	44
9. Simple Freeze-Dry Apparatus	48
10. Simple Freeze-Dry Apparatus with Refrigerated Condenser	49
11. Configuration of Continuous-Coil Refrigeration System	50
12. Detachable Refrigeration Coil	51
13. Flare Connector	51
14. Refrigeration Configuration in Large Specimen Chamber	52
15. Schematic of Large Two-Compressor Freeze-Dry System with Crossover Valves	52
16. Refrigerated Condenser Core	53
17. Refrigerated Condenser Core	53
18. Cell-Drying Apparatus	54
19. Flying Squirrel, <i>Glaucomys volans</i>	57
20. Pigmy Marmoset, <i>Chuchicos pygmaea</i>	57
21. Soldering Aid in Use	58
22. Cedar Waxwing, <i>Bombycilla cedrorum</i>	59
23. Yellow-breasted Chat, <i>Icteria virens virens</i>	59
24. Barred Owl, <i>Strix varia</i>	60
25. Yellow-shafted Flicker, <i>Colaptes auratus</i>	60
26. Sharp-shinned Hawk, <i>Accipiter striatus velox</i>	61
27. Robin, <i>Turdus migratorius</i>	61
28. Starlings, <i>Sturnus vulgaris vulgaris</i>	62
29. Central American Rattlesnake, <i>Crotalus</i>	64
30. Tabulated Tortoise	65
31. Alligator, <i>Alligator mississippiensis</i>	65
31. Monitor Lizard, <i>Varanus salvator</i>	66
33. Mounted Amphibian	66
34. Pumpkinseed, <i>Lepomis gibbosus</i>	67
35. Freshwater Fish	68
36. Marine Worms	69
37. Hermit Crab, <i>Paguridae</i>	70
38. Whale Louse, <i>Bathynomus giganteus</i>	70
39. Blue Crab, <i>Callinectes sapidus</i>	71
40. Ghost Crab	71
41. Sponge	72
42. Heat-Forming Glass Eyes	73
43. Beading Glass Chips	73
44. Heat-Formed Beads	74

45. Eye Master	75
46. Master Mold	75
47. Placing Heat-Formed Glass Pupils	76
48. Pouring Plastic Eyes	76
49. Finished Plastic Eyes	77
50. Pouring Master for Vacuum-Former	77
51. Vacuum-Form Master	78
52. Vacuum-Former	78
53. Vacuum-Formed Pouring Shell	79
54. Eyes Poured in Plastic Shell	79
55. Black and Yellow Argiope, <i>Argiope aurantia</i>	80
56. Arachnid	81
57. Insect Larvae	81
58. Praying Mantis	82
59. Simple Histology Apparatus	83
60. Baboon Lungs	84
61. Sectioned Baboon Heart	85
62. Human Stomach	86
63. Human Kidney Section	87
64. Stained Section, Human Brain	90
65. Human Liver Tissue	91
66. Bovine Eye Tissue	92
67. Human Spleen Tissue	92
68. Human Kidney, Polycystic	93
69. Human Lung Tissue	94
70. Human Lung Tissue	95
71. Human Fetus	96
72. Human Brain Section, Medulary	97
73. Section of Human Liver	98
74. Human Brain Tissue	99
75. Human Brain Section	99
76. Cancer Growth in Humeral Bone	101
77. Enlarged Heart (Arteriosclerosis)	102
78. Xerograph of Enlarged Heart	103
79. Section of Heart Valve	103
80. Section Through Heart Valve	104
81. Lymphosarcoma Involving the Right Ventrical	105
82. Brain Tumor and Skull	106
83. Critical-Point Drying Appartus	109
84. Hope Washing Apparatus	111
85. <i>Anathron dethrix</i> Kornicker, 1975	112
86. <i>Spinacopia bisetula</i> Kornicker, 1969	113
89. <i>Cymbicopia hanseni</i> (Brady, 1898)	114
88. <i>Philomedes lofthousae</i> Kornicker, 1975	114
89. <i>Skogsbergiella spinifera</i> (Skogsberg, 1920)	115
90. <i>Vargula subantarctica</i> Kornicker, 1975	116
91. Protozoa, <i>Suctorio</i>	117
92. Protozoa, <i>Suctorio</i>	118

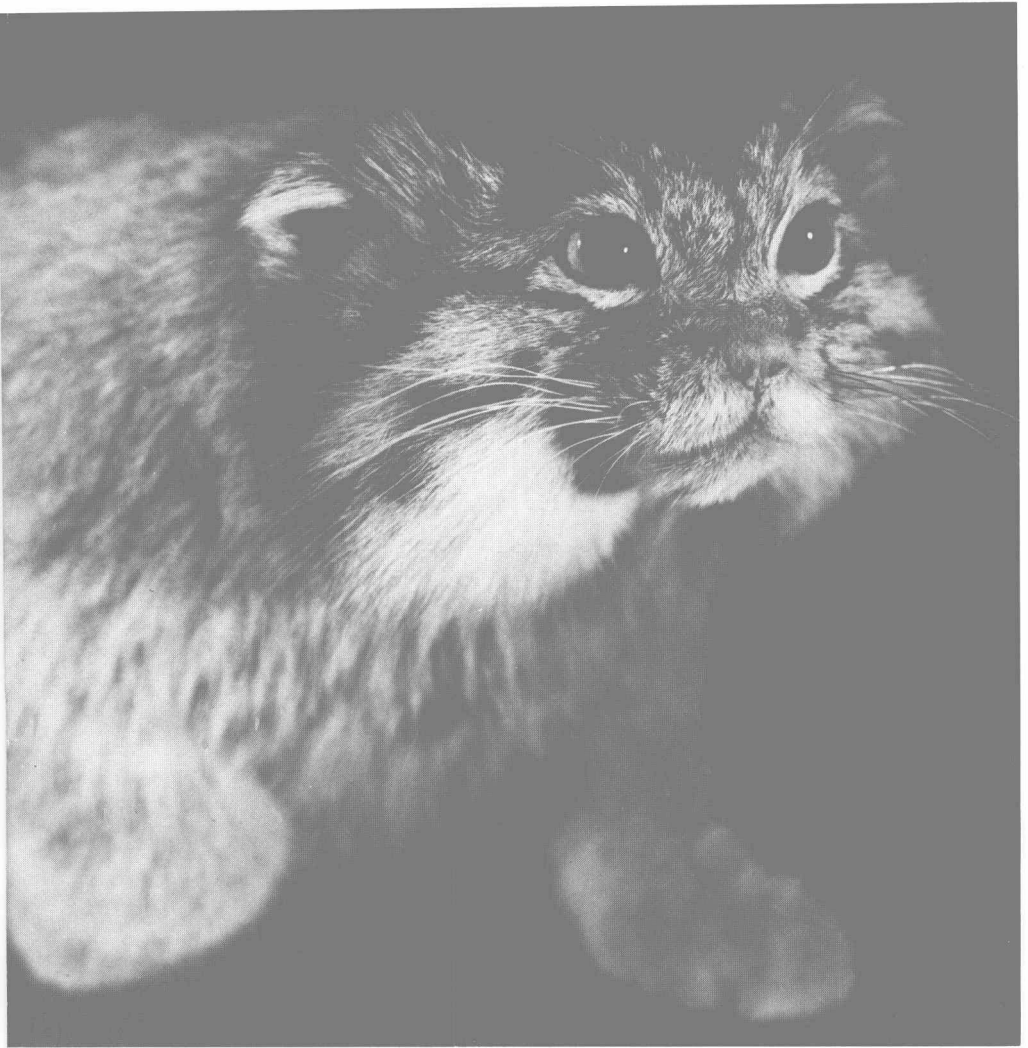
93. <i>Desmodora</i>	119
94. <i>Enoplus</i>	119
95. <i>Enoplus</i>	120
96. <i>Eurystomina</i>	120
97. Brain Body	121
98. Blood Vessels in Brain	122
99. Capillary in Brain	123
100. Almanac from the <i>Bertrand</i>	125
101. Items Taken from Blockade Runners . . .	126
102. Fragment of Ship Timber	127
103. Bovine Tissue (Control)	129
104. Bovine Tissue	129
105. Freezing Variations During Electrolytic Conduction	130
106. Surface-to-Mass Ratio, Mass-to-Time Ration	133
107. Bovine Muscle Drying Sample #1	134
108. Bovine Muscle Drying Sample #2	135
109. Bovine Muscle Drying Sample #3	136
110. Bovine Muscle Drying Sample #4	137
111. Bovine Muscle Drying Sample #5	138
112. Bovine Muscle Drying Sample #6	139
113. Baboon Lungs Drying Rate	140
114. Human Stomach Drying Rate	141
115. Human Fetus Drying Rate	142
116. Human Thyroid Drying Rate	143
117. Human Heart Drying Rate	144
118. Human Cardiac-Material Drying Rate	145
119. Human Lung Drying Rate	146
120. Human Brain Section Drying Rate	147
121. Human Brain Section Drying Rate	148
122. Human Brain Section Drying Rate	149
123. Brain, Complete Drying Rate	150
124. Eastern Chipmunk Drying Rate	151
125. Cave Bats Drying Rate	152
126. Cave Bats Drying Rate	153
127. Meadow Vole Drying Rate	154
128. Mourning Dove Drying Rate	155
129. Barred Owl Drying Rate	156
130. Red-bellied woodpecker Drying Rate	157
131. Slate-colored Junco Drying Rate	158
132. Ruby-throated Hummingbird Drying Rate	159
133. Virginia Rail Drying Rate	160
134. Eastern Chipping Sparrow Drying Rate	161
135. Hooded Warbler Drying Rate	162
136. Philadelphia Verio Drying Rate	163
137. Brown Creeper Drying Rate	164
138. Cedar Waxwing Drying Rate	165
139. Indigo Bunting Drying Rate	166
140. Cottonmouth Water Moccasin Drying Rate	167

141. Green Water Snake Drying Rate	168
142. Octopus Drying Rate	169
143. Skate Drying Rate	170
144. Hermit Crab Drying Rate	171
145. Almanac Drying Rate	172

Tables

1. Freezing Mixtures	23
2. Thermodynamic Properties of Water	26
3. Pressure and Mean Free Path	30
4. Vacuums	32
5. Conversion Multipliers	32
6. Metric Conversion	38
7. Thermal Conductivity of Various Insulating Materials	45
8. Calibration for Thermocouples	47
9. Wire Gauges and Sizes	56
10. Formulation of Neutral Formalin	89
11. Thermal Conductivity of Gases	107
12. Temperature Conversion	173
13. Underwriters' Laboratories Classification of Comparative Life Hazards of Gases and Vapors	174
14. Temperature-Pressure Table for Various Refrigerants	175

Freeze-Drying Biological
Specimens:
A Laboratory Manual



Frontispiece: Figure 1. Pallas Cat, Felis manul.

Freeze-Drying Biological Specimens: A Laboratory Manual

By Rolland O. Hower
Office of Exhibits Central
Smithsonian Institution
Washington, D.C.

Introduction by
R. H. Harris
British Museum of Natural History
London, England

Copyright © 1979 Smithsonian Institution. All rights reserved.

Library of Congress Cataloging in Publication Data

Hower, Rolland O.

Freeze-Drying Biological Specimens: A Laboratory Manual.

Bibliography: p.

Includes indexes.

1. Freeze-drying. 2. Biological specimens—

Collection and preservation. I. Title.

QH324.9.C7H68 579'.2 78-10750

ISBN 0-87474-532-2

Cover: Freeze-dried specimen of Maxwell screech owl (*Otus maxwellii*)

Photograph by James A. Mahoney, Chief, Office of Exhibits Central,
Smithsonian Institution.

All other photographs by the author.

Typeface: Times Roman

Paper: Paloma 70 lb. text, coated matte

Designed by Natalie Babson

Printed by Eastern Press, Inc.

Fondly dedicated to the memory of
John E. Anglim
who shared the dream,
supported the research, encouraged the author,
and was most of all a friend.

CONTENTS

Foreword by Paul N. Perrot	13
Preface: A Brief Survey of Freeze-Dry Research	15
Introduction: by R. H. Harris	19
I. FUNDAMENTALS OF FREEZE-DRY	23
Freezing Biological Tissue	23
Thermodynamics of Water: Its Properties in Various States	25
Vapor Pressure and Sublimation	26
The Freeze-Dry Concept	28
Transfer of Water Molecules to Specimen Surface	29
II. ENGINEERING SPECIFICATIONS	31
Basic Requirements of a Freeze-Dry System	31
Vacuum Pump	31
Rotary Oil Pump	32
Rotary Piston Pump	33
Gas Ballasting	34
Oil Diffusion Pump	35
Calculating Pump Capacity	35
Sealing Oil	37
Vacuum-Line Dimensions	37
Vacuum Measurement	39
Basic Concepts of Refrigeration	42
Refrigerants	43
Expansion Valves	44
Thermal Insulation	45
Temperature Measurement	46
Simple Freeze-Dry Apparatus in a Chest-Type Freezer	47
Condenser	48

Freeze-Dry System with a Refrigerated Specimen Chamber	49
Large Freeze-Dry System	51
Apparatus for Freeze-Drying Cell Material	53
 III. BIOLOGICAL APPLICATIONS	 55
Preparation of Zoological Specimens	55
Wire for Mounting Specimens	55
Small Mammals	56
Birds	58
Collecting Zoological Specimens	58
Labels and Notes	62
Cleaning Feathers	62
Stopping Blood Flow in Fresh Zoological Specimens	63
Reptiles	63
Fishes	66
Marine Invertebrates	68
Eyes	72
Insects and Insect Larvae	74
Killing the Larvae	82
Killing Arachnids	82
Fresh Anatomical Specimens	83
Preserved Biological Specimens	88
Processing a Human Fetus	89
Processing a Human Brain	96
Pathology Specimens	100
Microanalytic Study of Inflated Whole Lungs	100
Protecting Specimens from Insects	107
Shipping Frozen Biological Specimens	108
Preparation of Specimens for Scanning Electron Microscopy	109
Ostracods	110
Metal Coating	112
Nematodes	117
Human Brain Material	121
Preservation of Marine Archeological Materials	124
Salted Beef	124
Electrolytic Conduction During Freezing	125
 IV. APPENDIX A Drying Rate Charts	 131
APPENDIX B Temperature Conversion Tables	173
 V. GLOSSARY	 177
 VI. REFERENCES	 183
 VII. INDEX A Authors	 190
INDEX B Subject	191